



UNIVERSITI PUTRA MALAYSIA

**ASSESSMENT OF GREEN-LIPPED MUSSEL *PERNA VIRIDIS*
(LINNAEUS) AS A BIOMONITORING AGENT OF CADMIUM,
COPPER, MERCURY, LEAD AND ZINC FOR THE WEST COAST OF
PENINSULAR MALAYSIA**

YAP CHEE KONG

FSAS 2003 59

**ASSESSMENT OF GREEN-LIPPED MUSSEL *PERNA VIRIDIS* (LINNAEUS) AS
A BIOMONITORING AGENT OF CADMIUM, COPPER, MERCURY, LEAD
AND ZINC FOR THE WEST COAST OF PENINSULAR MALAYSIA**

By

YAP CHEE KONG

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirement for the Degree of Doctor
of Philosophy**

February 2003



DEDICATION

**This doctoral thesis is especially dedicated to the following most patient persons in my
life who made the impossible, possible.**

**My parents,
brother and sisters,
and my fiancée (Miss Choh Mew Seong).**

This doctoral degree is also dedicated to the departure memory of my grandmother.

Thanks to God.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

ASSESSMENT OF GREEN-LIPPED MUSSEL *PERNA VIRIDIS* (LINNAEUS) AS A BIOMONITORING AGENT OF CADMIUM, COPPER, MERCURY, LEAD AND ZINC FOR THE WEST COAST OF PENINSULAR MALAYSIA

By

YAP CHEE KONG

February 2003

Chairman : Associate Professor Ahmad Bin Ismail, Ph.D.

Faculty : Science and Environmental Studies

Sediment samples (46 sites) and green-lipped mussels *Perna viridis* (L.) (19 sites) from the west coast of Peninsular Malaysia were sampled between 1998 and 2001 and were analyzed for Cd, Cu, Hg, Pb and Zn. The mean levels of the metals in the west coast (offshore : intertidal) sediments were $0.75 \pm 0.06 : 0.86 \pm 0.11$ $\mu\text{g/g}$ dry weight (dw) for Cd, $4.27 \pm 0.36 : 29.22 \pm 8.84$ $\mu\text{g/g}$ dw for Cu, $38.87 \pm 2.93 : 60.10 \pm 9.47$ ng/g dw for Hg, $17.36 \pm 0.99 : 23.32 \pm 3.39$ $\mu\text{g/g}$ dw for Pb and $37.22 \pm 2.01 : 84.64 \pm 12.62$ $\mu\text{g/g}$ dw for Zn. The metal levels in the soft tissues (ST) of *P. viridis* were found to be 0.25-1.35 $\mu\text{g/g}$ dw for Cd, 6.31-20.21 $\mu\text{g/g}$ dw for Cu, 20.00-152.00 ng/g dw for Hg, 1.27-8.76 $\mu\text{g/g}$ dw for Pb and 53.82-135.50 $\mu\text{g/g}$ dw for Zn. All these metal levels were relatively low in comparison with previously published regional data. In general, the metal levels were higher in the intertidal sediment than in the offshore sediment. Geochemical studies in the sediments revealed that about 58.6 : 57.7% for Cd, 53.3 : 46.3% for Cu, 72.6 : 54.3% for Pb and 34.5 : 48.7% for Zn, in the offshore and intertidal sediments, respectively, were most likely due to anthropogenic sources. Localized elevations of

heavy metals in a few locations indicated that the offshore and intertidal areas of the west coast of Peninsular Malaysia were likely to have received anthropogenic metals.

By using the protein level allozyme approach, the genetic differentiation among the different geographical populations of this species fell within the range for conspecific populations. Since *P. viridis* populations are sedentary, widely distributed in the west coastal area of Peninsular Malaysia and have low genetic differentiation, the species generally is a good biomonitoring agent for heavy metals in the area. The mussel *P. viridis* collected from the field also showed that the metal concentrations in its total ST were positively and significantly ($P < 0.05$) correlated with Cd, Cu and Pb in the environment as represented by the sediment samples.

The suitability of *P. viridis* as a biomonitoring agent for heavy metals was experimentally studied. The results of the laboratory experiments showed that the ST of *P. viridis* was readily capable of accumulating heavy metals especially Cd, Pb and Hg to elevated levels. Depuration studies also showed that the metal levels in different STs were significantly ($P < 0.05$) correlated with those of the seawater. By using endpoints mortality, filtration rate (FR) and condition index (CI), *P. viridis* was found to be a sensitive but tolerant organism to Cd, Cu, Pb and Zn. The byssus (BYS) of *P. viridis* was found to be a sensitive biomonitoring tool for Zn whereas its total shell was a good biomonitoring material for Cd and Pb. Allozyme polymorphism of *P. viridis* was found to be a potential biomarker for metal contamination. Simple indicators (aerial exposure and shell deformities of *P. viridis*) for heavy metal pollution were also identified.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENILAIAN KUPANG *PERNA VIRIDIS* (LINNAEUS) SEBAGAI AGEN
PENUNJUK BIOLOGI KEPADA KADMIUM, KUPRUM, RAKSA, PLUMBUM
DAN ZINK UNTUK PANTAI BARAT SEMENANJUNG MALAYSIA**

Oleh

YAP CHEE KONG

Februari 2003

Pengerusi : Profesor Madya Ahmad Bin Ismail, Ph.D.

Fakulti : Sains dan Pengajian Alam Sekitar

Sedimen sampel (46 kawasan) dan kupang *Perna viridis* (19 kawasan) di pantai barat Semenanjung Malaysia telah disampel di antara 1998 dan 2001 dan dianalisis untuk Cd, Cu, Hg, Pb dan Zn. Tahap logam berat tersebut di sedimen adalah (luar-pantai : pasang-surut) $0.75 \pm 0.06 : 0.86 \pm 0.11$ $\mu\text{g/g}$ berat kering (bk) untuk Cd, $4.27 \pm 0.36 : 29.22 \pm 8.84$ $\mu\text{g/g}$ bk untuk Cu, $38.87 \pm 2.93 : 60.10 \pm 9.47$ ng/g bk untuk Hg, $17.36 \pm 0.99 : 23.32 \pm 3.39$ $\mu\text{g/g}$ bk untuk Pb dan $37.22 \pm 2.01 : 84.64 \pm 12.62$ $\mu\text{g/g}$ bk untuk Zn. Tisu lembut *P. viridis* didapati di antara $0.25\text{-}1.35$ $\mu\text{g/g}$ bk untuk Cd, $6.31\text{-}20.21$ $\mu\text{g/g}$ bk untuk Cu, $20.00\text{-}152.00$ ng/g bk untuk Hg, $1.27\text{-}8.76$ $\mu\text{g/g}$ bk untuk Pb dan $53.82\text{-}135.50$ $\mu\text{g/g}$ bk untuk Zn. Kesemua tahap logam tersebut adalah rendah berbanding dengan data di sekitar rantau ini. Secara keseluruhannya, tahap logam berat di sedimen pasang-surut adalah lebih tinggi daripada sedimen luar-pantai. Kajian geokimia menunjukkan bahawa $58.6 : 57.7\%$ untuk Cd, $53.3 : 46.3\%$ untuk Cu, $72.6 : 54.3\%$ untuk Pb dan $34.5 : 48.7\%$ untuk Zn, bagi sedimen luar-pantai dan pasang-surut, adalah disebabkan oleh aktiviti manusia. Namun begitu, tahap logam berat yang tertumpu di lokasi yang tertentu

menunjukkan bahawa pantai barat Semenanjung Malaysia telah menerima logam berat hasil daripada aktiviti manusia di lautan dan daratan.

Dengan kaedah alozim protein, differensi genetik di antara pelbagai populasi geografi didapati di dalam julat populasi 'conspecific'. Disebabkan *P. viridis* bertaburan luas di kawasan persisiran pantai barat Semenanjung Malaysia, mempunyai corak hidup yang tetap dan tahap differensi genetik yang rendah, spesies ini adalah agen penunjuk biologi yang baik kepada kawasan tersebut. Kupang *P. viridis* yang disampel di dalam lapangan juga menunjukkan logam berat di dalam tisu lembutnya berkolerasi positif dan signifikan ($P < 0.05$) dengan Cd, Cu dan Pb di persekitarannya yang diwakili sampel sedimen.

Melalui eksperimen-eksperimen yang dijalankan di dalam makmal, tisu lembut *P. viridis* didapati berkebolehan menumpuk logam berat yang tinggi terutamanya Cd, Pb dan Hg. Ini menunjukkan kesesuaian *P. viridis* sebagai agen penunjuk biologi kepada logam berat tersebut. Kajian depurasi juga menunjukkan tahap logam di pelbagai tisu lembut berkolerasi secara signifikan ($P < 0.05$) dengan air laut. Dengan menggunakan mortaliti, kadar filtrasi dan 'condition index', *P. viridis* didapati adalah sensitif tetapi ia adalah organisma yang bertoleransi tinggi terhadap Cd, Cu, Pb dan Zn. Kajian ini juga mendapati bisus *P. viridis* adalah lebih sensitif sebagai organ penunjuk kepada Zn manakala keseluruhan cangkerang *P. viridis* adalah bahan penunjuk biologi yang baik kepada Cd dan Pb. Alozim polimorfisma bagi *P. viridis* adalah penunjuk biologi yang berpotensi bagi pencemaran logam berat manakala penunjuk biologi yang mudah

(pendedahan udara dan cangkerang *P. viridis* yang abnormal) bagi pencemaran logam berat telah dikenalpasti.

ACKNOWLEDGMENTS

I am most indebted and grateful to my supervisor Assoc. Prof. Dr. Ahmad Ismail who had guided me along the way towards the completion of this doctoral degree. He had given me a lot of constructive comments, advice and ideas. With his support and motivation, my research activities were conducted efficiently and on time.

Also, I would like to extend my sincere gratitude to Prof. Dr. Tan Soon Guan who has guided me for the protein allozyme study of my work and also taught me a lot on how to write good scientific papers. His time, kind and constant encouragement are very much appreciated. My appreciation also goes to Dr. Hishamuddin Omar for his kind support. Thanks to the Japan International Cooperation Agency (JICA) and Malacca Straits Research and Development Centre (MASDEC) for organizing the scientific expedition cruises in the Straits of Malacca. The isozyme work was supported in part by IRPA grant 01-02-04-0074 which is also highly appreciated. My thanks are also extended to the Fisheries Institute at Batu Maung, Penang, for providing the strain of *Isochrysis galbana*.

My thanks are also extended to Abu Hena, Khai Khin, Nik, Sem, Kak Rosniza, Hamid Rezaei, Ali Mashinchian, Mr. Hidir, Kok Kuan and all my friends who indirectly contributed so much towards this achievement.



TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGMENTS	viii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xviii
LIST OF FIGURES	xxvi
LIST OF ABBREVIATIONS	xxxiii
CHAPTER	
1 INTRODUCTION	1
1.1 Objectives of Study	4
2 LITERATURE REVIEW	6
2.1 Biomonitoring of West Coast of Peninsular Malaysia	7
2.2 Sources of Heavy Metals in Malaysia	13
2.3 Biomonitoring Agents for Heavy Metals	17
2.4 Advantages of Using Intertidal Molluscs as Biomonitoring Agents	19
2.5 Criteria for A Good Biomonitoring Agent	22
2.6 Heavy Metal Biomonitoring Studies	23
2.6.1 Regional Studies	23
2.6.2 Malaysian Studies	24
2.7 Green-Lipped Mussel <i>Perna viridis</i> as A Biomonitoring Agent for Heavy Metals in Malaysia	29
2.8 Reported Studies About <i>Perna viridis</i> as A Biomonitoring Agent	34
2.9 Genetic Information about the Biomonitoring Agents	36
2.10 Changes of Genetic Structures of Biomonitoring Agents Due to Heavy Metal Contamination	40
2.11 Intertidal Stresses to Marine Mussels	42
2.11.1 Salinity Stress	43
2.11.2 Wave Action	44
2.11.3 Aerial Exposure	46
2.12 Metabolic Pathways of Mussels During Submersion and Emersion	46
2.13 Mussel Byssus	51
2.13.1 Composition of A Mussel Byssus	51
2.13.2 Byssal Formation and Function of A Mussel Byssus	54
2.14 A Filter-Feeder Mussel	55
2.14.1 Mechanisms of A Filter-Feeding Activity in Mussels	56
2.14.2 Roles of the Filter-Feeder Bivalves in the Intertidal Waters	63
2.15 Uptake and Bioavailability and Toxicities of Heavy Metals	67
2.16 Importance of Laboratory Study	73



3	MATERIALS AND METHODS	76
3.1	Study Area	76
3.1.1	Sampling Locations of Offshore and Intertidal Sediments of the West Coast of Peninsular Malaysia	76
3.1.2	Sampling Locations of <i>Perna viridis</i>	82
3.2	Sample Storage	86
3.2.1	Sediment	86
3.2.2	Mussels	86
3.2.2	Seawater	87
3.3	Sample Preparation	87
3.3.1	Sediment	87
3.3.2	Soft Tissues of <i>Perna viridis</i>	87
3.3.3	Shells of <i>Perna viridis</i>	88
3.3.4	Byssus of <i>Perna viridis</i>	89
3.4	Sample Digestion/Extraction	89
3.4.1	Sediment: Total Concentrations of Cd, Cu, Pb and Zn	89
3.4.2	Sediment: Total Concentration of Hg	90
3.4.3	Speciations of Cd, Cu, Pb and Zn in Sediment Sample: Sequential Extraction Technique	90
3.4.4	Soft Tissues, Shell and Byssus of <i>Perna viridis</i> : Total Concentrations of Cd, Cu, Pb and Zn	94
3.4.5	Soft Tissues, Shell and Byssus of <i>Perna viridis</i> : Total Concentration of Hg	94
3.5	Determinations of Cd, Cu, Pb and Zn	94
3.6	Determination of Hg	95
3.7	Quality Assurance / Quality Control Activities	96
3.7.1	Cleaning Procedures	96
3.7.2	Blank: Checking for External Contamination	96
3.7.3	Quality Control Sample	97
3.7.4	Certified Reference Material	97
3.7.5	Standard Addition Technique	99
3.8	Maintenance of Instruments (AAS and Hg Analyzer)	99
3.9	Determination of Organic Matter Content	100
3.10	Determinations of Ammonia, Phosphate and Nitrate in Seawater	100
3.10.1	Ammonium (NH_4^+) Analysis	100
3.10.2	Nitrate (NO_3^-) Analysis	101
3.10.3	Phosphate (PO_4^{2-}) Analysis	102
3.11	Determination of Total Protein	102
3.12	Laboratory Experimental Study	103
3.12.1	Acclimatization of <i>Perna viridis</i> under Laboratory Conditions	103
3.12.2	Experimental Conditions of the Test Seawater	106
3.12.3	Culture of Brown Alga <i>Isochrysis galbana</i>	106
3.13	Filtration Rate of <i>Perna viridis</i>	108
3.13.1	Determination of Filtration Rate	109
3.13.2	Percentage of Filtration Rate Reduction	111
3.14	Toxicity Tests of Cd, Cu, Pb and Zn on <i>Perna viridis</i>	111

3.14.1 Physiological Response: Mortality (LC ₅₀)	111
3.14.2 Physiological Response: Filtration Rate (EC ₅₀)	113
3.15 Condition Index of <i>Perna viridis</i>	113
3.15.1 Determination of Condition Index	114
3.15.2 Percentage of Condition Index Reduction	114
3.16 Sublethal Metal Exposure on <i>Perna viridis</i>	114
3.17 Accumulation and Depuration Studies on <i>Perna viridis</i>	115
3.17.1 Rates of Accumulation and Depuration of Metals	115
3.17.2 Percentage of Metal Reduction	116
3.17.3 Bioconcentration Factors	116
3.18 Sampling of Fecal Materials of <i>Perna viridis</i>	117
3.19 Pollution Indices	119
3.19.1 Pollution Load Index	119
3.19.2 Metal Pollution Index	120
3.20 Allozyme Analysis	121
3.21 Statistical Analytical Procedures	127
3.21.1 Coefficient of Variation	128
3.21.2 Cluster Analysis	129
3.21.3 Student's t and Student-Newman-Keuls Tests	130
3.21.4 Pearson's Product Moment Correlation Coefficient	130
3.21.5 Probit Analysis	131
3.21.6 Allozyme Data: Heterozygosity, Genetic Distance/Identity and <i>F</i> -Statistics	131
4 RESULTS AND DISCUSSION	133
4.1 Heavy Metal Levels of Offshore and Intertidal Sediments of the West Coast of Peninsular Malaysia	133
4.1.1 EFLE Fractions of Cd, Cu, Pb and Zn	133
4.1.2 'Acid-Reducible' Fractions of Cd, Cu, Pb and Zn	137
4.1.3 'Oxidisable-Organic' Fractions of Cd, Cu, Pb and Zn	142
4.1.4 'Resistant' Fractions of Cd, Cu, Pb and Zn	146
4.1.5 Overall Distributions of the Geochemical Fractions of Cd, Cu, Pb and Zn	150
4.1.6 A Comparison of Total Concentrations of Cd, Cu, Pb and Zn with Regional Data	156
4.1.7 A Comparison of Total Concentration of Hg with Regional Data	167
4.1.8 A Comparison of Northern, Central and Southern Regions of the Straits of Malacca	172
4.1.9 Proposed Sediment Quality Guidelines for Cd, Cu, Hg, Pb and Zn	176
4.1.10 Studies on Correlation Matrices between Heavy Metals in Sediment	178
4.1.11 Summary	187
4.2 Levels of Cd, Cu, Hg, Pb and Zn in Total Soft Tissue of <i>Perna viridis</i>	190

4.2.1 Heavy Metal Levels of <i>Perna viridis</i> in Different Sampling Sites	190
4.2.2 Pollution Indices	200
4.2.3 Comparisons of Cd, Cu, Pb and Zn with Reported Data	203
4.2.4 A Comparison of Hg with Reported Data	207
4.2.5 Heavy Metal Levels from Public Health Point of View	210
4.2.6 Summary	216
4.3 Physiological Response to Heavy Metals:	217
<i>viridis</i>	
4.3.1 Effects of Amount of Seawater	217
4.3.2 Effects of Algal Concentrations	219
4.3.3 Effects of Mussel Shell Length (Body Size)	221
4.3.4 Effects of Heavy Metals After 2 and 28 Days of Metal Exposure	222
4.3.5 Toxicity Tests of Cd, Cu, Pb and Zn	223
4.3.6 Summary	229
4.4 Physiological Response to Heavy Metals:	230
4.4.1 Sensitivity of Different Size (Shell Length) Groups	230
4.4.2 Toxicity Tests of Cd, Cu, Pb and Zn	233
4.4.3 A Comparison between Values of LC ₅₀ (Mortality) and EC ₅₀ (Filtration Rate)	242
4.4.4 Summary	243
4.5 Physiological Response to Heavy Metals:	245
<i>viridis</i>	
4.5.1 Effects of 2 and 28 Days of a Single Metal Exposure to Condition Index of <i>Perna viridis</i>	245
4.5.2 Effects of 1 and 3 Days of Multiple Metal Exposures to Condition Index	249
4.5.3 Relationships between Condition Index and Heavy Metals	253
4.5.4 Low Condition Index Values Due to High Levels of Nutrients (Ammonia, Phosphate and Nitrate) Released	257
4.5.5 Strategies to Overcome Affecting Factors on Condition Index- Nutritional and Reproductive States and Body Size of <i>Perna viridis</i>	260
4.5.6 Summary	263
4.6 Accumulation and Depuration Studies in Different Soft Tissues of <i>Perna viridis</i>	264
4.6.1 Experimental Results of Cd, Cu, Hg, Pb and Zn Accumulation and Depuration Studies	264
4.6.2 Ability to Accumulate Cd, Cu, Hg, Pb and Zn	274
4.6.3 Similar Patterns of Accumulation and Depuration of Cd, Cu, Hg, Pb and Zn in the Different Soft Tissues	284
4.6.4 Ability to Depurate the Accumulated Levels of Cd, Cu, Hg, Pb and Zn	292
4.6.5 Different Levels of Metals in Different Soft Tissues	298

4.6.6 Summary	311
4.7 Genetic Differentiation of <i>Perna viridis</i> Populations	312
4.7.1 Phenotypes of Loci	312
4.7.2 Genetic Variability within Populations	329
4.7.3 Conformation with Hardy-Weinberg Equilibrium	329
4.7.4 Genetic Differentiation	332
4.7.5 Genetic Similarity / Distance	334
4.7.6 General Discussion	335
4.7.7 Summary	340
4.8 Relationships between <i>Perna viridis</i> and Its Environment	341
4.8.1 Correlation Coefficients of Metals in Soft Tissue of <i>Perna viridis</i> and Seawater and Sediment	341
4.8.2 Summary	353
4.9 Relationships of Protein Allozyme Variation of <i>Perna viridis</i> and Heavy Metal Levels in the Sediment	354
4.9.1 Metal Levels of Mussels and Sediments from Contaminated and Uncontaminated Sites	354
4.9.2 Genetic Similarity / Distance of Contaminated and Uncontaminated Populations	356
4.9.3 Correlation Coefficients between Metal Pollution Indices and Protein Allozyme Variations of <i>Perna viridis</i>	362
4.9.4 Summary	366
4.10 Byssus of <i>Perna viridis</i> as A Biomonitoring Organ for Cd, Cu, Pb and Zn	367
4.10.1 Byssus Can Readily Accumulate Heavy Metals and the Metal Levels were Comparatively Higher than Those of Total Soft Tissue	367
4.10.2 Accumulation of Metals Found in Byssus was Mainly Due to Metabolic Pathways	371
4.10.3 Byssus as A Sensitive Biomonitoring Organ for Heavy Metals	376
4.10.4 Byssus as A Route of Metal Excretion	379
4.10.5 Summary	383
4.11 Shell of <i>Perna viridis</i> as A Biomonitoring Material for Cd, Cu, Pb and Zn	384
4.11.1 Shell Can Readily Accumulate Heavy Metals Especially Cd and Pb	384
4.11.2 Low Variabilities of Metals in the Shell Especially for Cd, Cu and Pb	390
4.11.3 Significant Associations of Cd, Cu, Pb and Zn within the Shell	391
4.11.4 Shell had Lower Rates of Accumulation and Different Patterns of Depuration than Those of the Total Soft Tissue	393
4.11.5 Periostracum as A Biomonitoring Material for Heavy Metals	396

4.11.6 Summary	399
4.12 Simple Indicators of Metal Contamination	400
4.12.1 Duration of Aerial Exposure of <i>Perna viridis</i> as An Estimate of Cu and Cd Contaminations	400
4.12.2 Shell Deformity of <i>Perna viridis</i> as An Indicator of Metal Contamination in Coastal Waters of Peninsular Malaysia	409
4.12.3 Summary	419
5 GENERAL DISCUSSION AND CONCLUSION	420
REFERENCES	428
VITA	476

LIST OF TABLES

Table		Page
2.1	Sources o heavy metal inputs in the west coast of Peninsular Malaysia according to DOE (1999).	16
2.2	Potential candidates as biomonitoring agents for various metals in the Malaysian intertidal environments.	27
2.3	A comparison of field and laboratory studies.	74
3.1	Sampling dates, research vessels used and number of stations covered during the four cruises of the Straits of Malacca Scientific Expeditions.	76
3.2	Positions, estimates of depth and distances from the shore and places nearest to the sampling locations for the Straits of Malacca Scientific Expeditions.	79
3.3	Descriptions of the types of offshore sediments sampled during the first, second, third and fourth cruises of the Straits of Malacca Scientific Expeditions.	80
3.4	Positions, sampling dates, sediment types and descriptions of sampling sites for the intertidal sediments collected from the west coast of Peninsular Malaysia.	81
3.5	Sampling dates and shell lengths of <i>Perna viridis</i> analyzed and descriptions of sampling sites in the coastal waters of Peninsular Malaysia.	84
3.6	A comparison of the analytical results of the Certified Reference Material for Soil with the certified concentrations of Cd, Cu, Hg, Pb and Zn.	98
3.7	Compositions of Conway and culture solutions used for the experimental culture of <i>Isochrysis galbana</i> .	107
3.8	Concentrations of measured and nominal values of Cd, Cu, Pb and Zn in the test seawater used for the toxicity tests by using filtration rate a physiological response in <i>Perna viridis</i> .	112
3.9	Concentrations of measured and nominal values of Cd, Cu, Pb and Zn in the test seawater used for the toxicity tests by using filtration rate a physiological response in <i>Perna viridis</i> .	113

tissue of *Perna viridis* collected from coastal waters off Peninsular Malaysia.

4.12	Results of t-test comparisons of Cd, Cu, Pb and Zn levels in the total soft tissue of <i>Perna viridis</i> between two sampling periods for selected sampling sites.	198
4.13	A comparison of reported concentrations of Cd, Cu, Pb and Zn in <i>Perna viridis</i> from regional studies with the present results and those from other studies done in Malaysia.	204
4.14	A comparison of concentrations of Cd, Cu, Pb and Zn in <i>Perna viridis</i> with other biological samples reported from Malaysia and Singapore.	206
4.15	A comparison of Hg level in <i>Perna viridis</i> with hairs and other biological samples reported from regional studies.	208
4.16	Guidelines on heavy metals for food safety set by different countries.	211
4.17	Guidelines on Hg levels used in other countries.	215
4.18	Filtration rate and percentage of filtration rate reduction of <i>Perna viridis</i> exposed to different levels of Cu in seawater and two different size groups of <i>P. viridis</i> .	222
4.19	Filtration rate and percentage of filtration rate reduction of <i>Perna viridis</i> exposed to Cd, Pb and Zn after days 2 and 28.	222
4.20	Filtration rates and percentages of filtration rate reduction of <i>Perna viridis</i> exposed to different levels of Cd, Cu, Pb and Zn in seawater.	225
4.21	Results of EC ₅₀ by Probit analysis on the toxicities of Cd, Cu, Pb and Zn by using filtration rate of <i>Perna viridis</i> as the physiological response.	225
4.22	Rates of accumulation of two significant size groups of <i>Perna viridis</i> .	230
4.23	Results of LC ₅₀ by Probit analysis on the toxicities of Cd, Cu, Pb and Zn by using mortality of <i>Perna viridis</i> .	232
4.24	A comparison of reported LC ₅₀ values of Cd, Cu, Pb and Zn by using mortality of <i>Perna viridis</i> .	234
4.25	Results of the levels of Cd, Cu, Pb and Zn in seawater and total soft	236

tissue of *Perna viridis* and their bioconcentration factor values after 24 hours of exposure to different concentrations of the metals.

4.26	A comparison of LC ₅₀ and EC ₅₀ values that used <i>Perna viridis</i> as a test organism.	243
4.27	Condition index of <i>Perna viridis</i> after 2 and 28 days of exposures to Cd 0.50 mg/l, Pb 1.50 mg/l and Zn 1.00 mg/l.	247
4.28	Metal levels and rate of metal accumulation in total soft tissue of <i>Perna viridis</i> after 2 and 28 days of exposures to Cd 0.50 mg/l, Pb 1.50 mg/l and Zn 1.00 mg/l.	247
4.29	Condition index of <i>Perna viridis</i> after 1 and 3 days of exposures to Cd 1.00 mg/l, Cu 0.15 mg/l and Cd 1.00 mg/l + Cu 0.15 mg/l.	249
4.30	Metal levels and rate of metal accumulation in total soft tissue of <i>Perna viridis</i> after 1 and 3 days of exposures to Cd 1.00 mg/l, Cu 0.15 mg/l and Cd 1.00 mg/l + Cu 0.15 mg/l.	250
4.31	A comparison of Cd, Cu, Pb and Zn concentrations and condition index values of <i>Perna viridis</i> between K. Perlis and Kg. Tg. Batu.	253
4.32	Pearson's correlation coefficients between condition index and heavy metal concentrations in total soft tissue of <i>Perna viridis</i> .	254
4.33	Factors that affect the condition index of <i>Perna viridis</i> and strategies used to overcome these factors during laboratory and field studies.	261
4.34	Levels of Cd in different soft tissues and bioconcentration factors in <i>Perna viridis</i> during accumulation and depuration periods of Cd 1.21 mg/l.	265
4.35	Rates of accumulation and depuration of Cd in different soft tissues in <i>Perna viridis</i> exposed to 1.21 mg/l of Cd in seawater.	265
4.36	Levels of Cu in different soft tissues and bioconcentration factors in <i>Perna viridis</i> during accumulation and depuration periods of Cu 0.20 mg/l.	267
4.37	Rates of accumulation and depuration of Cu in different soft tissues in <i>Perna viridis</i> exposed to 0.2 mg/l of Cu in seawater.	267
4.38	Levels of Hg in different soft tissues and bioconcentration factors in <i>Perna viridis</i> during accumulation and depuration periods of Hg 0.10 mg/l.	269

4.39	Rates of accumulation and depuration of Hg in different soft tissues in <i>Perna viridis</i> exposed to 0.10 mg/l of Hg in seawater.	269
4.40	Levels of Pb in different soft tissues and bioconcentration factors in <i>Perna viridis</i> during accumulation and depuration periods of Pb 2.95 mg/l.	271
4.41	Rates of accumulation and depuration of Pb in different soft tissues in <i>Perna viridis</i> exposed to 2.95 mg/l of Pb in seawater.	271
4.42	Levels of Zn in different soft tissues and bioconcentration factors in <i>Perna viridis</i> during accumulation and depuration periods of Zn 1.95 mg/l.	273
4.43	Rates of accumulation and depuration of Zn in different soft tissues in <i>Perna viridis</i> exposed to 1.95 mg/l of Zn in seawater.	273
4.44	Ratios of maximum to minimum values of Cd concentration in <i>Perna viridis</i> reported from regional field studies and those found from the present work.	275
4.45	Ratios of maximum to minimum values of Cu concentration in <i>Perna viridis</i> reported from regional field studies and those found from the present work.	276
4.46	Ratios of maximum to minimum values of Hg concentration in <i>Perna viridis</i> reported from regional field studies and those found from the present work.	277
4.47	Ratios of maximum to minimum values of Pb concentration in <i>Perna viridis</i> reported from regional field studies and those found from the present work.	278
4.48	Ratios of maximum to minimum values of Zn concentration in <i>Perna viridis</i> reported from regional field studies and those found from the present work.	279
4.49	Occurrences of metallothionein-like proteins in other marine bivalve species.	297
4.50	Phenotypes of fourteen loci in eight mussel populations.	313
4.51	Allele frequencies of fourteen loci in eight mussel populations.	330
4.52	A summary of chi-square tests for deviations from Hardy-Weinberg Equilibrium.	332

4.53	<i>F</i> -statistics values for fourteen loci of eight populations of <i>Perna viridis</i> .	333
4.54	Wright's (1978) hierarchical <i>F</i> -statistics of genetic differentiation for eight <i>Perna viridis</i> populations grouped into two and three regions.	333
4.55	Nei's (1978) genetic identity and genetic distance for eight populations of <i>Perna viridis</i> from Peninsular Malaysia.	334
4.56	A comparison of F_{ST} values reported for different molluscs species and populations with the present result.	337
4.57	A comparison of genetic distance values reported for different invertebrate species and populations with the present result.	339
4.58	Values of Nei's (1978) genetic identity and genetic distance for four populations of <i>Perna viridis</i> .	356
4.59	Values of observed heterozygosity, expected heterozygosity, deviations of expected heterozygosity and χ^2 tests of goodness of fit to the Hardy-Weinberg model for four mussel populations.	359
4.60	A comparison of metal pollution index and percentage of polymorphic loci and mean heterozygosity at fourteen loci in four mussel populations.	362
4.61	Pearson's correlation coefficients among pairs of percentage of polymorphic loci and metal pollution index values in sediment and that in <i>Perna viridis</i> .	363
4.62	Heavy metal concentrations in byssus and total soft tissue, and ratios of metals in byssus/total soft tissue of <i>Perna viridis</i> sampled from the field.	368
4.63	A comparison of byssus/total soft tissue ratios for Cd, Cu, Pb and Zn concentrations of other mussel species with the present results.	369
4.64	Pearson's correlation coefficients of Cd, Cu, Pb and Zn between byssus and total soft tissue of <i>Perna viridis</i> and between byssus and seawater.	373
4.65	A comparison among levels of Cd, Cu, Pb and Zn in different soft tissues of <i>Perna viridis</i> before metal exposure, at the end of exposure and at the end of depuration.	376

4.66	Percentages of total protein in the byssus, periostracum, total soft tissue and the nacreous shell layer of <i>Perna viridis</i> .	377
4.67	Rates of accumulation and depuration of Cd, Cu, Pb and Zn in byssus and total soft tissue of <i>Perna viridis</i> .	378
4.68	Rates of depuration of Cd, Zn and Pb in fecal materials, byssus and total soft tissue in <i>Perna viridis</i> during depuration period.	381
4.69	Bioconcentration factor values of Cd, Cu, Pb and Zn in byssus and total soft tissue of <i>Perna viridis</i> .	381
4.70	Cd concentrations in total soft tissue and shell and ratios of shell/ST of <i>Perna viridis</i> collected from the field.	385
4.71	Cu concentrations in total soft tissue and shell and ratios of shell/ST of <i>Perna viridis</i> collected from the field.	385
4.72	Pb concentrations in total soft tissue and shell and ratios of shell/ST of <i>Perna viridis</i> collected from the field.	386
4.73	Zn concentrations in total soft tissue and shell and ratios of shell/ST of <i>Perna viridis</i> collected from the field.	386
4.74	A comparison of ratios of shell/total soft tissue for Cd, Cu, Pb and Zn concentrations of other molluscs species with those of <i>Perna viridis</i> .	388
4.75	A comparison of coefficient of variations for Cd, Cu, Pb and Zn concentrations in total soft tissue and total shell of <i>Perna viridis</i> .	390
4.76	Pearson's correlation coefficients between heavy metal concentrations in the shell and total soft tissue of <i>Perna viridis</i> .	392
4.77	Rates of accumulation and depuration of Cd, Cu, Pb and Zn in shell and total soft tissue of <i>Perna viridis</i> .	393
4.78	Bioconcentration factor values for periostracum, nacreous layer and total soft tissue of <i>Perna viridis</i> during laboratory experiment study.	398
4.79	Concentrations of Cu and Cd in total soft tissue of <i>Perna viridis</i> during different periods of laboratory experimental exposures to Cu, Cd and a combination of Cu and Cd.	402
4.80	Condition index of <i>Perna viridis</i> during different periods of laboratory experimental exposures to Cu, Cd and a combination of	403

Cu and Cd.

4.81	Frequencies of shell deformity of all types in <i>Perna viridis</i> from 15 sampling sites of Peninsular Malaysia.	410
4.82	Pearson's correlation coefficients between frequency of shell deformity and Cd, Cu, Pb and Zn in soft tissue and shell of <i>Perna viridis</i> and sediment of sampling site.	416
5.1	A comparison of recommended criteria fulfilled by <i>Perna viridis</i> based on field and laboratory experiment studies.	423
5.2	A summary of the proposed characteristics fulfilled by the physiological responses of <i>Perna viridis</i> used to test its tolerance and sensitivity to heavy metals.	425